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9. Erickson, J.W., and Quintero, J.J. (2007). Indirect effects of ploidy suggest X chromosome dose, not the X: A ratio, signals sex in Drosophila. PLoS Biol. *5*, e332.
10. Madl, J.E., and Herman, R.K. (1979). Polyploids and sex determination in *Caenorhabditis elegans*. Genetics *93*, 393–402.
11. Akerib, C.C., and Meyer, B.J. (1994). Identification of X chromosome regions in *Caenorhabditis elegans* that contain sex-determination signal elements. Genetics *138*, 1105–1125.
12. Hodgkin, J., Zellan, J.D., and Albertson, D.G. (1994). Identification of a candidate primary sex determination locus, fox-1, on the X chromosome of *Caenorhabditis elegans*. Development *120*, 3681–3689.
13. Nicoll, M., Akerib, C.C., and Meyer, B.J. (1997). X-chromosome-counting mechanisms that determine nematode sex. Nature *388*, 200–204.
14. Carmi, I., Kopczynski, J.B., and Meyer, B.J. (1998). The nuclear hormone receptor SEX-1 is an X-chromosome signal that determines nematode sex. Nature *396*, 168–173.
15. Gladden, J.M., and Meyer, B.J. (2007). A ONECUT homeodomain protein communicates X chromosome dose to specify *Caenorhabditis elegans* sexual fate by repressing a sex switch gene. Genetics *177*, 1621–1637.
16. Powell, J.R., Jow, M.M., and Meyer, B.J. (2005). The T-box transcription factor SEA-1 is an autosomal element of the X: A signal that determines *C. elegans* sex. Dev. Cell *9*, 339–349.
17. Gladden, J.M., Farboud, B., and Meyer, B.J. (2007). Revisiting the X: A signal that specifies *Caenorhabditis elegans* sexual fate. Genetics *177*, 1639–1654.
18. Miller, L.M., Plenefisch, J.D., Casson, L.P., and Meyer, B.J. (1988). xol-1: a gene that controls the male modes of both sex determination and X chromosome dosage compensation in *C. elegans*. Cell *55*, 167–183.
19. Rhind, N.R., Miller, L.M., Kopczynski, J.B., and Meyer, B.J. (1995). xol-1 acts as an early switch in the *C. elegans* male/hermaphrodite decision. Cell *80*, 71–82.
20. Avner, P., and Heard, E. (2001). X-chromosome inactivation: counting, choice and initiation. Nat. Rev. Genet. *2*, 59–67.

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Axon Outgrowth: Motor Protein Moonlights in Microtubule Sliding

Neurons develop from small, spherical precursors into the largest, most asymmetric of all metazoan cells by extending thin axonal processes over enormous distances. Although the forces for this extension have been unclear, recent work shows that the initial axonal extension may involve an unexpected mechanism: sliding of microtubules, driven by a motor protein previously thought to be deployed only in organelle transport.

Peter J. Hollenbeck and Daniel M. Suter

Neurons are the largest, most asymmetric and specialized of all metazoan cells, yet they start life as small, roughly spherical precursors. Their development and differentiation involve the initial establishment of long neuritic processes, referred to as axons and dendrites, and the subsequent extension of axons in particular over enormous distances — up to 1 meter in humans. The source and nature of the forces driving this axonal elongation have been studied for decades but are not fully understood. Now, work by Lu *et al.* [1], recently published in *Current Biology*, indicates that the force for initial neurite outgrowth may involve an unexpected mechanism: sliding of microtubules, driven by a motor protein, kinesin-1, previously thought to be deployed only in organelle transport.

The three major types of cytoskeletal filaments — microtubules, actin filaments and neurofilaments — are essential structural components of normal axons, supporting in various ways their establishment, growth, maintenance and resistance to mechanical stress. The contributions of microtubules and actin filaments to axonal elongation have been mainly studied in vertebrate neurons in cell culture, in the presence of various cytoskeletal drugs. Altogether these studies have revealed that microtubules appear to be more critical for the extension of axon-like processes, while actin filaments play a key role in growth cone motility and guidance [2–4]. Furthermore, neurite extension in culture can occur in the complete absence of actin filaments or microtubules [5] and axonal differentiation can occur in the absence of actin [6]. Nonetheless, other work has shown that dynamic microtubules are essential for growth cone guidance [7]. Thus, both microtubules and actin filaments are involved in various stages of axon formation; however, their respective contributions at each stage of development, as well as the role of filament assembly versus translocation in force generation under normal physiological conditions, remain unclear [8].

Lu *et al.* [1] studied the earliest stages of axon elongation using *Drosophila* neurons grown in culture. In these insect neurons, there are no neurofilaments, which clears the decks a bit, and gene and protein expression can be controlled more easily than in vertebrate cells. Previous work by this group had shown that microtubule sliding occurred in *Drosophila* S2 cells, a small, relatively rounded macrophage-like cell type [9]. There, they had used gene and protein knockdown techniques and the visualization of fluorescently tagged tubulin in live cells to show that kinesin-1-driven microtubule sliding was occurring. They furthermore predicted this phenomenon could provide force for cell-shape changes, such as process formation. In the new work, these authors confirmed that, as in vertebrates, *Drosophila* neurons could form and initially extend neurites *in vitro* in the absence of actin filaments, microtubule polymerization, or growth cone structures at their tips. To probe the nature of the initial extension of the axon, they visualized microtubules using a photoconversion approach and found that they actively slide relative to each other during the first few hours after cell plating, while sliding activity significantly declined in older neurons. Furthermore, sliding microtubule arrays extend to and appear to press against the expanding distal tip of young neurites. What motor protein powers this sliding? In fly kinesin-1 mutants and following dsRNA-induced reduction of kinesin-1,

they observed reduced microtubule bundling and sliding as well as reduced axon initiation and elongation. Together, these experiments provide evidence for a mechanism involving kinesin-1-mediated sliding of microtubules that drives the initial outgrowth of neurites from a rounded neuronal cell body.

The theme of neurons re-purposing a motor protein from rapid organelle transport to the sliding of filaments during initial neurite outgrowth should not come as a shock, since the slow axonal transport of both microtubules and neurofilaments is actually driven by 'fast' motors similar to those that drive organelle transport or mitosis, but operating a very small percentage of the time [10]. A number of 're-purposed' cytoplasmic motor proteins have been implicated in axonal microtubule transport, including cytoplasmic dynein in the anterograde direction [11–13], and kinesin-5 [14] and kinesin-12 [15] in the retrograde direction. The surprise of the study by Lu *et al.* [1] is that microtubule sliding driven by the plus-end motor kinesin-1, which delivers organelles and perhaps short polymers as 'cargo' to the distal axon, can also provide force generation for the initial protrusion of an axon.

In addition to microtubule sliding, microtubule growth by end polymerization has also been proposed to underlie axon elongation, particularly at the growth cone [16]. However, mild suppression of microtubule dynamics throughout the axon does not eliminate elongation, and the role of microtubule polymerization at axonal locations away from the growth cone remains uncertain [17]. Inconsistencies in the findings and conclusions of some earlier drug-based studies on this subject may result from differences in drug applications and culture systems. Although neurons *in vitro* can elaborate axons in the absence of a growth cone [6], navigation of the leading tip through the embryo to its proper target is utterly dependent on the growth cone and its dynamic, complex cytoskeleton and signal transduction machinery [18]. Since a large body of work indicates that both microtubule sliding and microtubule assembly can contribute to axonal elongation, it now seems essential to quantitatively assess the respective contribution of each process, for example, by precise live-cell imaging of microtubule plus ends and internal microtubule reference marks in the same axons.

The present study by Lu *et al.* [1] not only provides new insights into the underlying cytoskeletal mechanisms of neurite initiation but also raises many interesting questions that remain to be answered. Does a similar microtubule sliding mechanism drive initial neurite formation in vertebrate neurons? What is the contribution of microtubule sliding at different stages of axonal development? Since Lu *et al.* [1] show that microtubule sliding is downregulated very soon after axon initiation in young neurons, the mechanism for force generation for the bulk of axonal growth remains in question. How can kinesin-1 move microtubules in the anterograde direction? Translocating microtubule cargo anterogradely and thereby producing a force pushing against the membrane seems possible only if the adjacent microtubule-track for kinesin-1 is somehow immobilized within the cell. Furthermore, determining the level and significance of the force exerted on the plasma membrane by microtubule sliding versus assembly will require both sophisticated biophysical and imaging measurements as well as mathematical modeling. Lastly, the role of intrinsically generated forces versus extrinsically generated forces might be quite different between *in vitro* and *in vivo* axonal elongation. It is important to remember that, for most axons *in vivo*, massive stretching occurs during organismal growth, after axons have already been connected to their targets early in embryonic development. The enormous addition of axonal length and mass during this kind of growth could involve filament sliding, filament intercalation or other processes yet to be elucidated [8].

References

1. Lu, W., Fox, P., Lakonishok, M., Davidson, M.W., and Gelfand, V.I. (2013). Initial neurite outgrowth in Drosophila neurons is driven by kinesin-powered microtubule sliding. Curr. Biol. *23*, 1018–1023.
2. Marsh, L., and Letourneau, P.C. (1984). Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. J. Cell Biol. *99*, 2041–2047.
3. Bentley, D., and Toroian-Raymond, A. (1986). Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. Nature *323*, 712–715.
4. Letourneau, P.C., Shattuck, T.A., and Ressler, A.H. (1987). "Pull" and "push" in neurite elongation: observations of the effects of different concentrations of cytochalasin and taxol. Cell Motil. Cytoskel. *8*, 193–209.
5. Morris, R.L., and Hollenbeck, P.J. (1995). Axonal transport of mitochondria along microtubules and F-actin in living vertebrate neurons. J. Cell Biol. *131*, 1315–1326.
6. Ruthel, G., and Hollenbeck, P.J. (2000). Growth cones are not required for initial establishment of polarity or differential axon branch growth in cultured hippocampal neurons. J. Neurosci. *20*, 2266–2274.
7. Suter, D.M., Schaefer, A.W., and Forscher, P. (2004). Microtubule dynamics are necessary for SRC family kinase-dependent growth cone steering. Curr. Biol. *14*, 1194–1199.
8. Suter, D.M., and Miller, K.E. (2011). The emerging role of forces in axonal elongation. Prog. Neurobiol. *94*, 91–101.
9. Jolly, A.L., Kim, H., Srinivasan, D., Lakonishok, M., Larson, A.G., and Gelfand, V.I. (2010). Kinesin-1 heavy chain mediates microtubule sliding to drive changes in cell shape. Proc. Natl Acad. Sci. USA *107*, 12151–12156.
10. Brown, A. (2003). Axonal transport of membranous and nonmembranous cargoes: a unified perspective. J. Cell Biol. *160*, 817–821.
11. Dillman, J.F., 3rd, Dabney, L.P., and Pfister, K.K. (1996). Cytoplasmic dynein is associated with slow axonal transport. Proc. Natl. Acad. Sci. USA *93*, 141–144.
12. Ahmad, F.J., Echeverri, C.J., Vallee, R.B., and Baas, P.W. (1998). Cytoplasmic dynein and dynactin are required for the transport of microtubules into the axon. J. Cell Biol. *140*, 391–401.
13. He, Y., Francis, F., Myers, K.A., Yu, W., Black, M.M., and Baas, P.W. (2005). Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. J. Cell Biol. *168*, 697–703.
14. Myers, K.A., and Baas, P.W. (2007). Kinesin-5 regulates the growth of the axon by acting as a brake on its microtubule array. J. Cell Biol. *178*, 1081–1091.
15. Liu, M., Nadar, V.C., Kozielski, F., Kozlowska, M., Yu, W., and Baas, P.W. (2010). Kinesin-12, a mitotic microtubule-associated motor protein, impacts axonal growth, navigation, and branching. J. Neurosci. *30*, 14896–14906.
16. Bamburg, J.R., Bray, D., and Chapman, K. (1986). Assembly of microtubules at the tip of growing axons. Nature *321*, 788–790.
17. Yu, W., and Baas, P.W. (1995). The growth of the axon is not dependent upon net microtubule assembly at its distal tip. J. Neurosci. *15*, 6827–6833.
18. Lowery, L.A., and Van Vactor, D. (2009). The trip of the tip: understanding the growth cone machinery. Nat. Rev. Mol. Cell Biol. *10*, 332–343.

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